PCT/EP 03/06760

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Patentanmeldung Nr. Patent application No. Demande de brevet n°

02018907.2

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Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

Anmeldung Nr:
Application no.: 02018907.2
Demande no:

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Anmelder/Applicant(s)/Demandeur(s):

MBT Munich Biotechnology AG
Fraunhoferstrasse 10
82152 Martinsried
ALLEMAGNE

Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

Camptothecin-carboxylate formulations

In Anspruch genommene Priorität(en) / Priority(ies) claimed /Priorité(s) revendiquée(s)
Staat/Tag/Aktenzeichen/State/Date/File no./Pays/Date/Numéro de dépôt:

Internationale Patentklassifikation/International Patent Classification/
Classification internationale des brevets:

A61K31/00

Am Anmeldetag benannte Vertragstaaten/Contracting states designated at date of filing/Etats contractants désignées lors du dépôt:

AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LI LU MC NL PT SE SK TR

9. Claims

1. **A composition** comprising the carboxylate form of a camptothecin drug or a derivative thereof associated with at least one organic cationic molecule which has a positive net charge(cationic molecule), wherein said composition has a molar ratio of at least about 1:1 of an organic cationic molecule to camptothecin carboxylate and is substantially free of the lactone form of said drug or a derivative thereof.
2. The composition of claim 1, wherein said camptothecin carboxylate is selected from the ammonium, sodium or potassium salt of a camptothecin drug or a derivative thereof.
3. The composition of claim 1 to 2 wherein said organic cationic molecule is an amphiphile or a polymer.
4. The composition of any one of claims 1 to 3, wherein said cationic amphiphile is selected from lipids, lysolipids or pegylated lipids, preferably a cationic amphiphile with a tertiary amino or quaternary ammonium group such as N-[1-(2,3-diacyloxy)propyl]-N,N-dimethylamine or N-[1-(2,3-diacyloxy)propyl]-N,N,N-trimethyl ammonium.
5. The composition of any one of claims 1 to 4, wherein said polymer is a polyelectrolyte, acid such as polyallylamine or polyethylene imine, a polymeric sugar or a polyamino with a molecular weight between about 5 and 500 kDa.
6. The composition of any one of the claims 1 to 5, further comprising at least one amphiphile which has a negative and/or neutral net charge (anionic and/or neutral amphiphile).
7. The composition of any one of claims 1 to 6, wherein said anionic and/or neutral amphiphile is selected from sterols or lipids such as cholesterol, phospholipids, lysolipids, lysophospholipids, sphingolipids or pegylated lipids with a negative or neutral net change.
8. The composition of any one of the claims 1 to 7, wherein the neutral amphiphile is diacylphosphatidylcholine.
9. **A colloidal nanoaggregate** comprising a composition of any one of the claims 1 to 8.
10. The nanoaggregate of claim 9 having an overall positive charge.
11. The nanoaggregate of claim 9 or 10, comprising an excess of positively charged moieties of at least 20 %, preferably at least 30 % and most preferably at least 40 % in the outer molecular layer.

12. The nanoaggregate of any one of the claims 9 to 11, which is present as an emulsion droplet, a micelle, a liposome, a nanoparticle or a nanocapsule.
13. The nanoaggregate of any one of the claims 9 to 12, comprising about 1 to 50 mol % of the drug, preferably about 5 to 15 mol % of the drug.
14. The nanoaggregate of any one of the claims 9 to 13 which is a particle having a particle size ranging from about 5 nm to 5000 nm, preferably from 25 nm to 500 nm and more preferably from about 100 nm to 300 nm.
15. The nanoaggregate of any one of the claims 9 to 14, further comprising a cryoprotectant which is selected from a sugar or an alcohol or a combination thereof such as trehalose, maltose, sucrose, glucose, lactose, dextran, mannitol or sorbitol.
16. **A pharmaceutical preparation** comprising a pharmaceutically effective amount of the composition of any one of the claims 1 to 8 or a colloidal nanoaggregate of any one of the claims 9 to 15 together with a pharmaceutically acceptable carrier, diluent and/or adjuvant.
17. **A method of producing** the colloidal nanoaggregate of any one of the claims 9 to 15, comprising the steps of
    a) providing a camptothecin drug or derivative thereof, preferably as a salt and
    b) associating said drug with a cationic amphiphile which has a positive net charge (cationic amphiphile)
    c) and optionally at least one further amphiphile which has a positive, negative and/or neutral net charge (anionic and/or neutral amphiphile) forming a colloidal nanoaggregate.
18. The method of claim 17, wherein step b) and c) comprise forming said nanoaggregate by a homogenisation, a lipid film or by a solvent injection procedure.
19. **The use of** a pharmaceutical preparation of claim 16 for producing a medicament for treating and/or preventing a disease characterized by enhanced angiogenic activity.

Abstract

A composition comprising the carboxylate form of a camptothecin drug or a derivative thereof associated with at least one organic cationic molecule which has a positive net charge cationic molecule), wherein said composition has a molar ratio of at least about 1:1 of an organic cationic molecule to camptothecin carboxylate and is substantially free of the lactone form of said drug or a derivative thereof.

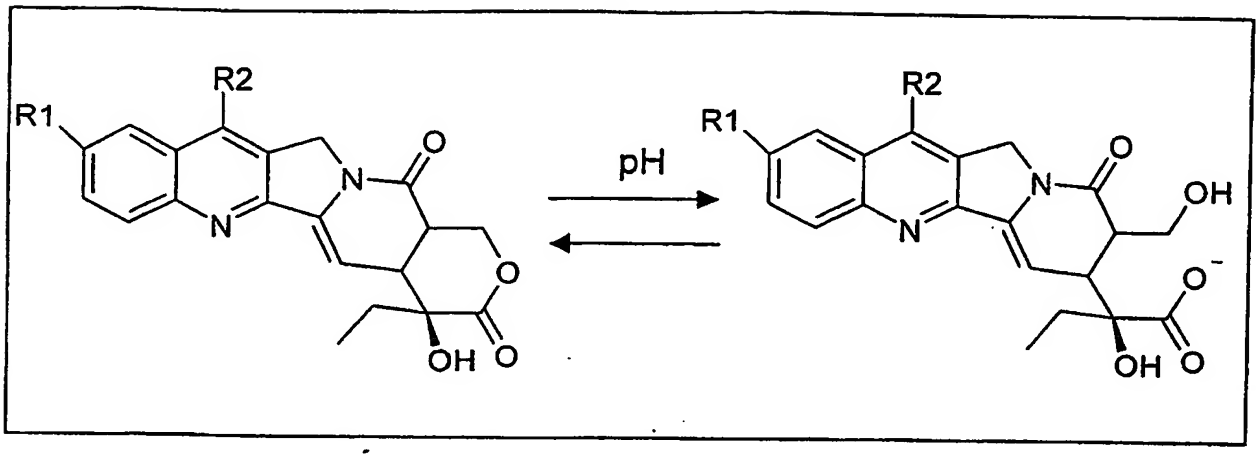


Fig. 1

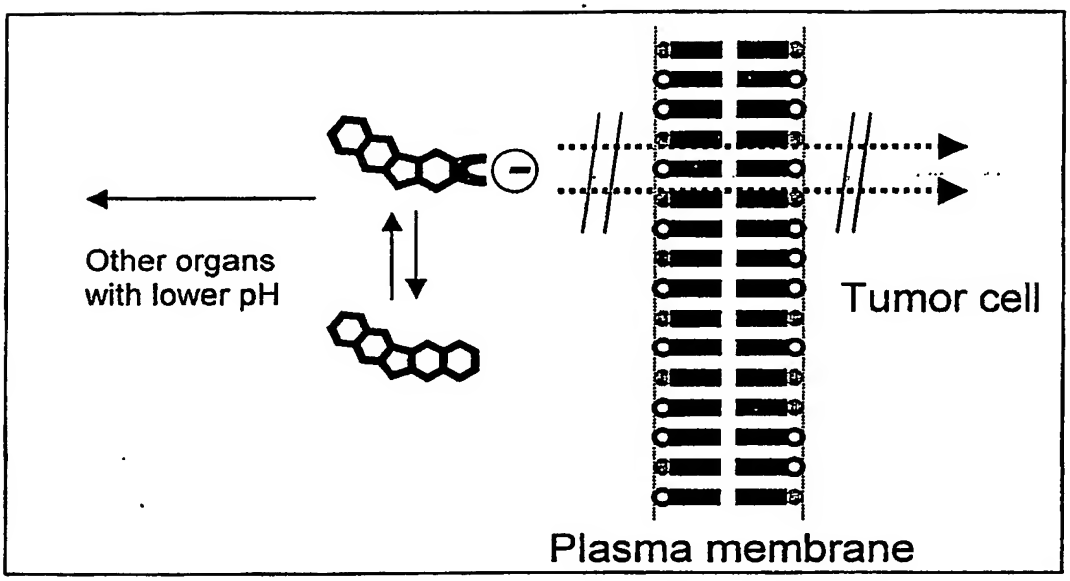


Fig. 2

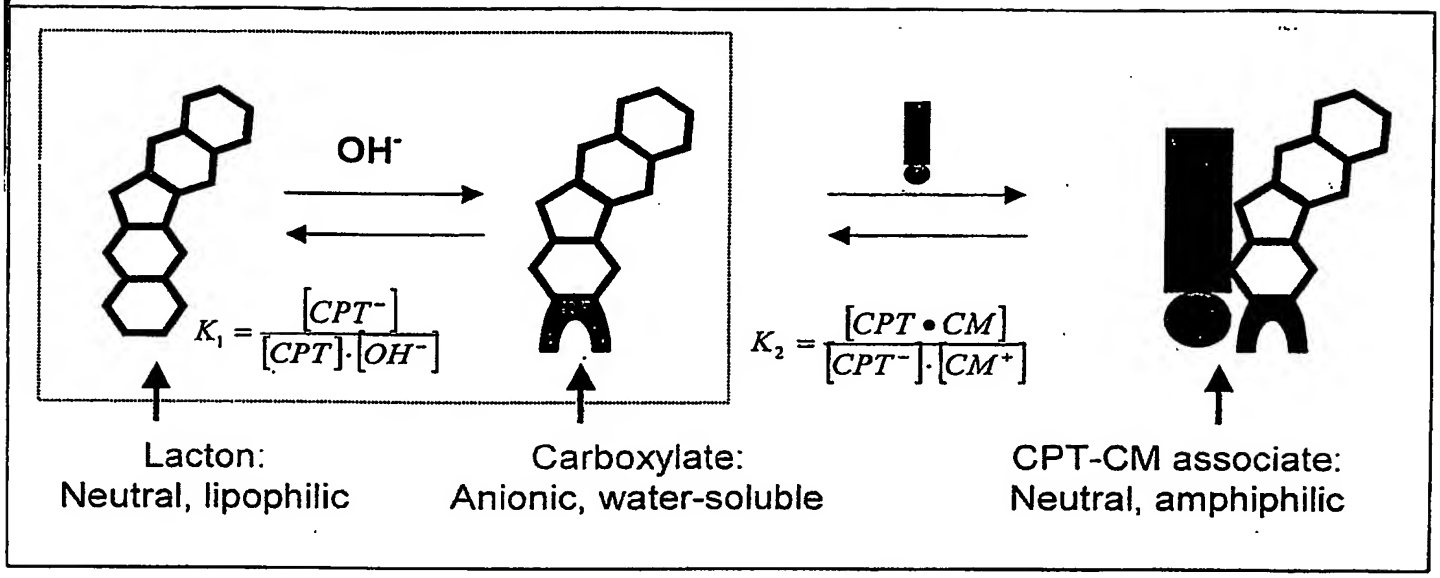

OH-
$K_1 = \frac{[CPT^-]}{[CPT] \cdot [OH^-]}$
$K_2 = \frac{[CPT \bullet CM]}{[CPT^-] \cdot [CM^+]}$
Lacton:
Neutral, lipophilic
Carboxylate:
Anionic, water-soluble
CPT-CM associate:
Neutral, amphiphilic

Fig. 3

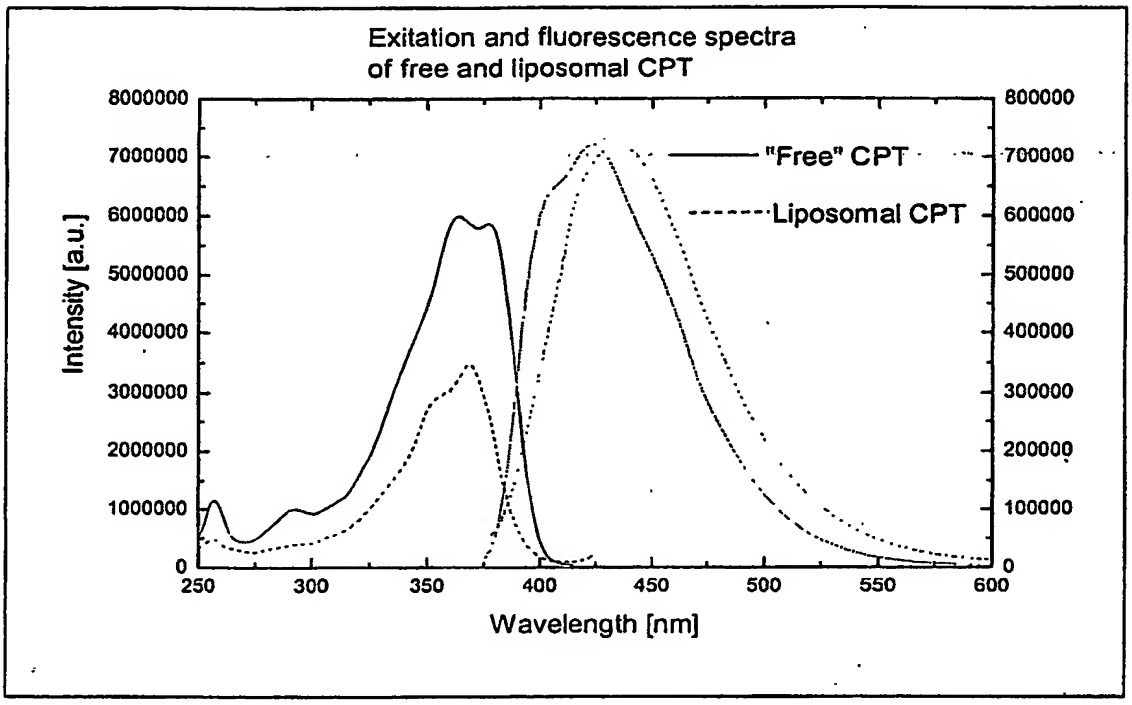

Exitation and fluorescence spectra
of free and liposomal CPT
"Free" CPT
Liposomal CPT
Intensity [a.u.]
Wavelength [nm]
8000000
7000000
6000000
5000000
4000000
3000000
2000000
1000000
0
800000
700000
600000
500000
400000
300000
200000
100000
0
250
300
350
400
450
500
550
600

Fig. 4

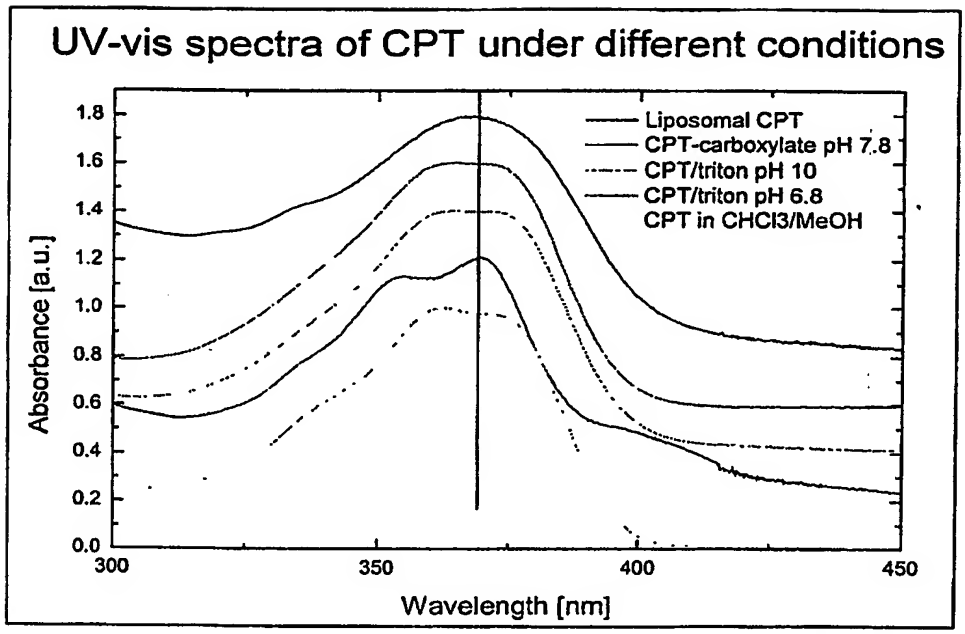
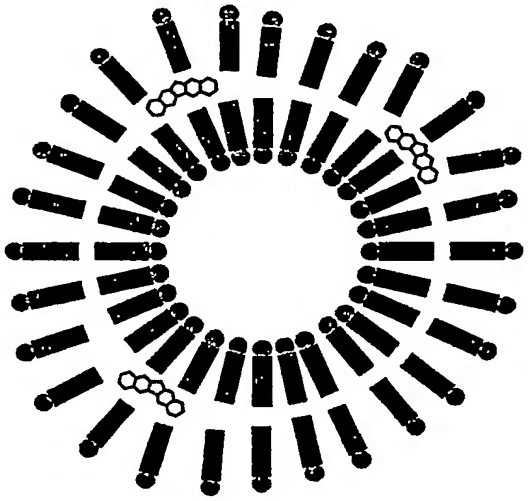
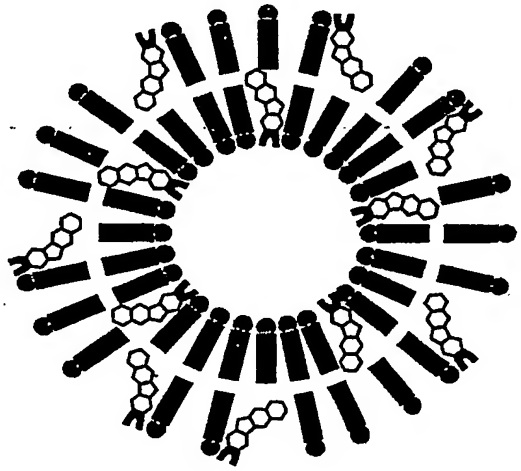

UV-vis spectra of CPT under different conditions
Liposomal CPT
CPT-carboxylate pH 7.8
CPT/triton pH 10
CPT/triton pH 6.8
CPT in CHCl3/MeOH
Absorbance [a.u.]
Wavelength [nm]
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
300
350
400
450

Fig. 5



Usually for lipophilic compounds (CPT-lacton):
Solubilization in the hydrocarbon region

•Only low drug/lipid ratio



New approach:
CM-CPT acts as colipid
It is an integral part of the liposome

•High drug/lipid ratio

Fig. 6

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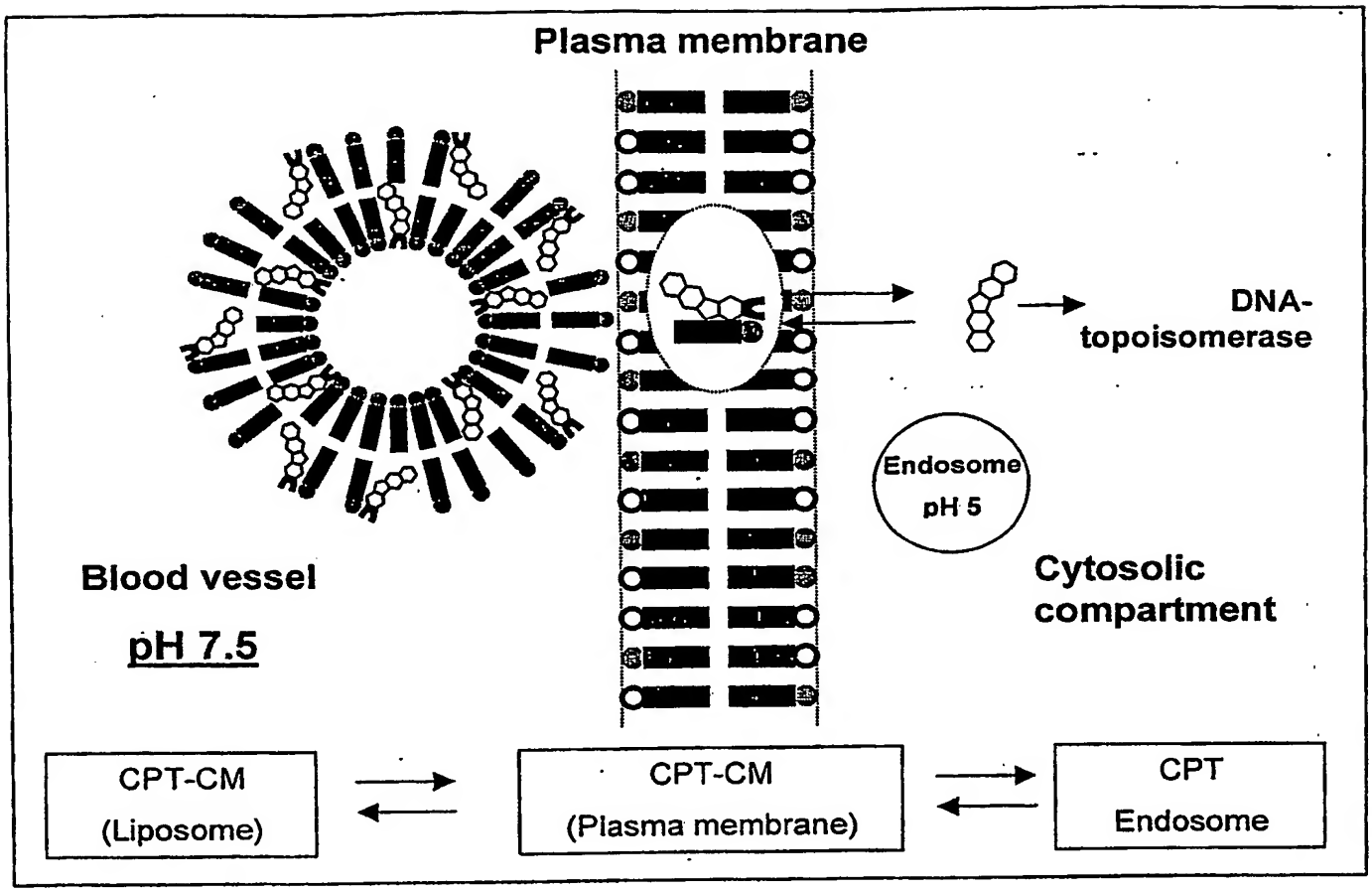
Plasma membrane
DNA-
topoisomerase
Endosome
pH 5
Blood vessel
pH 7.5
Cytosolic
compartment
CPT-CM
(Liposome)
CPT-CM
(Plasma membrane)
CPT
Endosome

Fig. 7

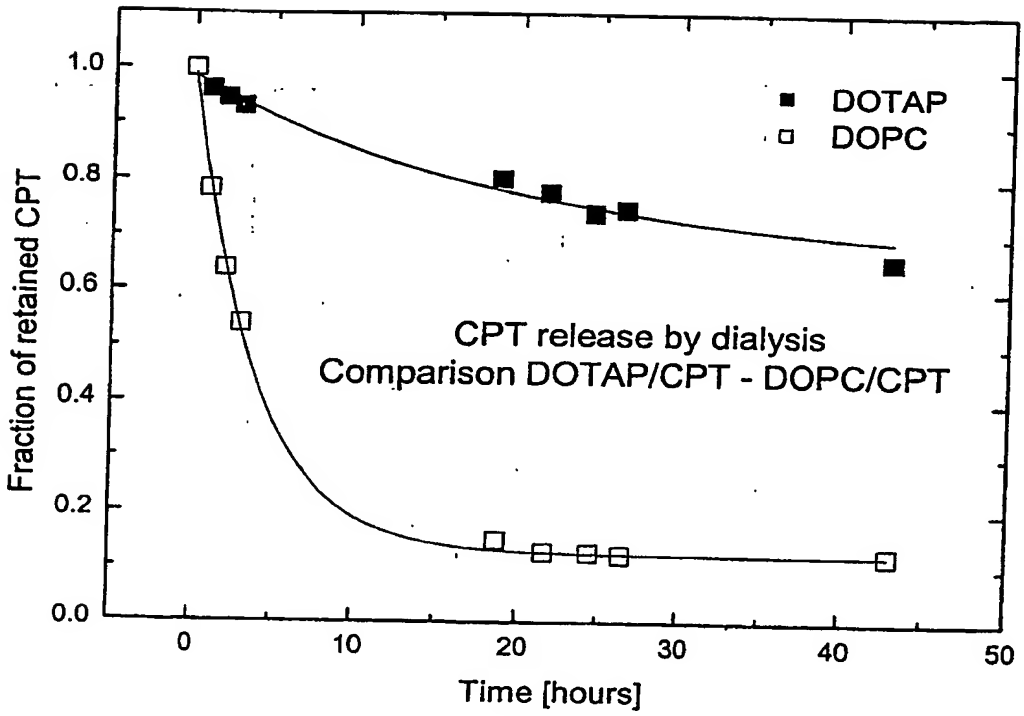
DOTAP
DOPC
CPT release by dialysis
Comparison DOTAP/CPT - DOPC/CPT
Fraction of retained CPT
Time [hours]
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50

Fig. 8

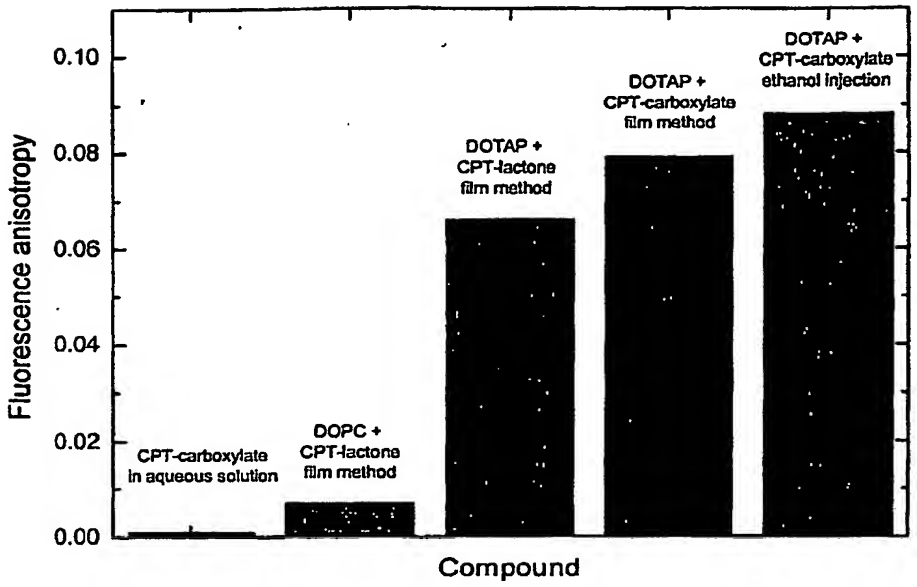
Fluorescence anisotropy
0.10
0.08
0.06
0.04
0.02
0.00
CPT-carboxylate in aqueous solution
DOPC + CPT-lactone film method
DOTAP + CPT-lactone film method
DOTAP + CPT-carboxylate film method
DOTAP + CPT-carboxylate ethanol injection
Compound

Fig 9

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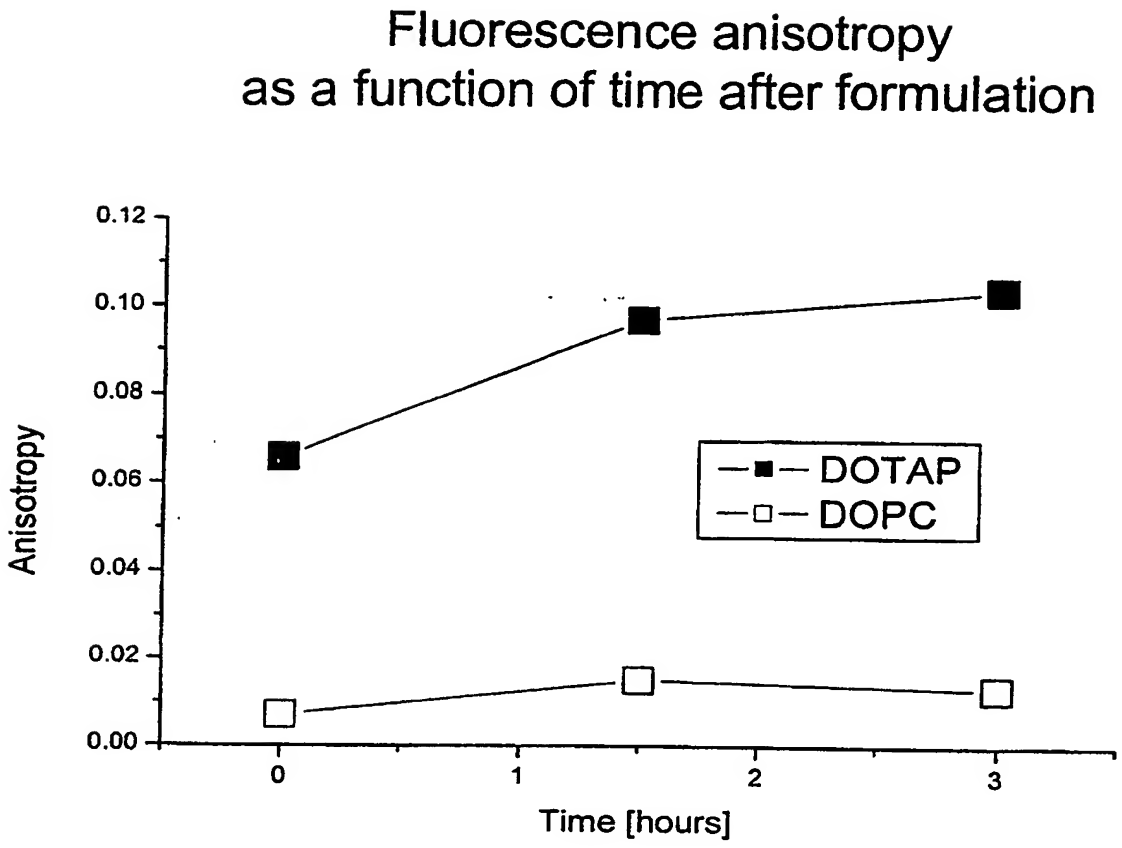
Fluorescence anisotropy
as a function of time after formulation
Anisotropy
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0
1
2
3
Time [hours]
DOTAP
DOPC

Fig. 10:

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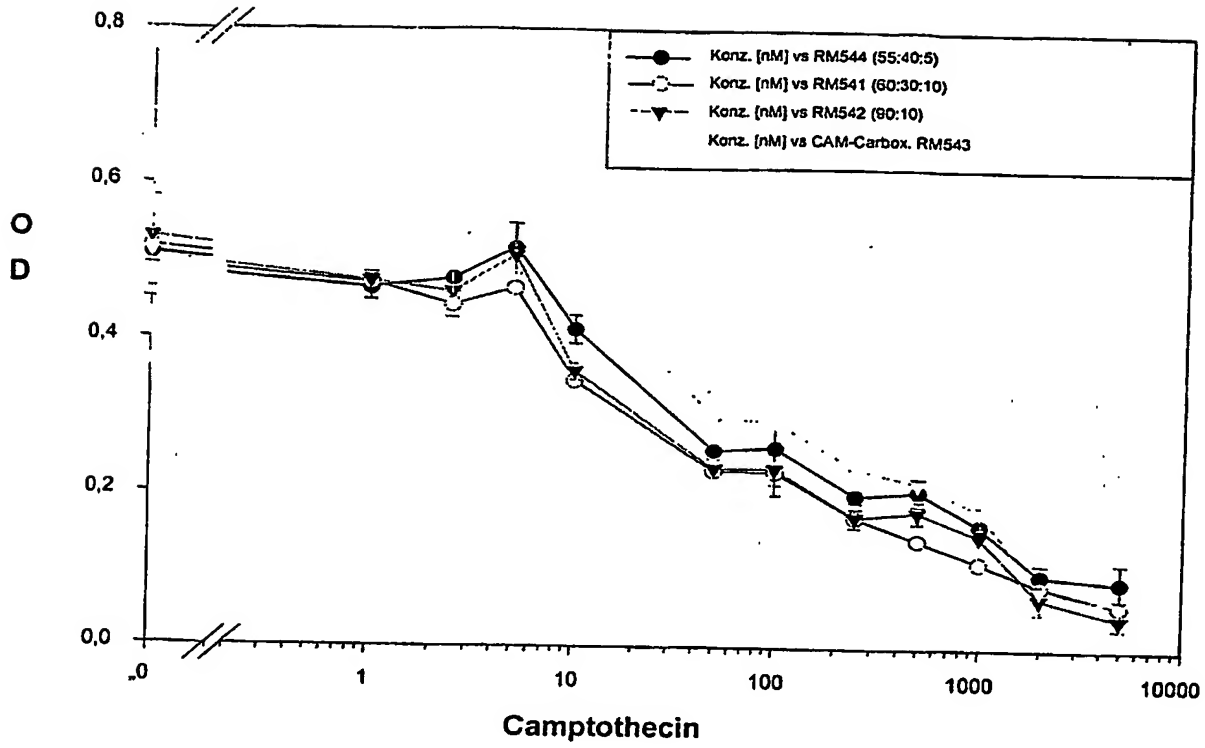
0,8
0,6
0,4
0,2
0,0
O
D
Konz. [nM] vs RM544 (55:40:5)
Konz. [nM] vs RM541 (60:30:10)
Konz. [nM] vs RM542 (90:10)
Konz. [nM] vs CAM-Carbox. RM543
0
1
10
100
1000
10000
Camptothecin

Fig.11

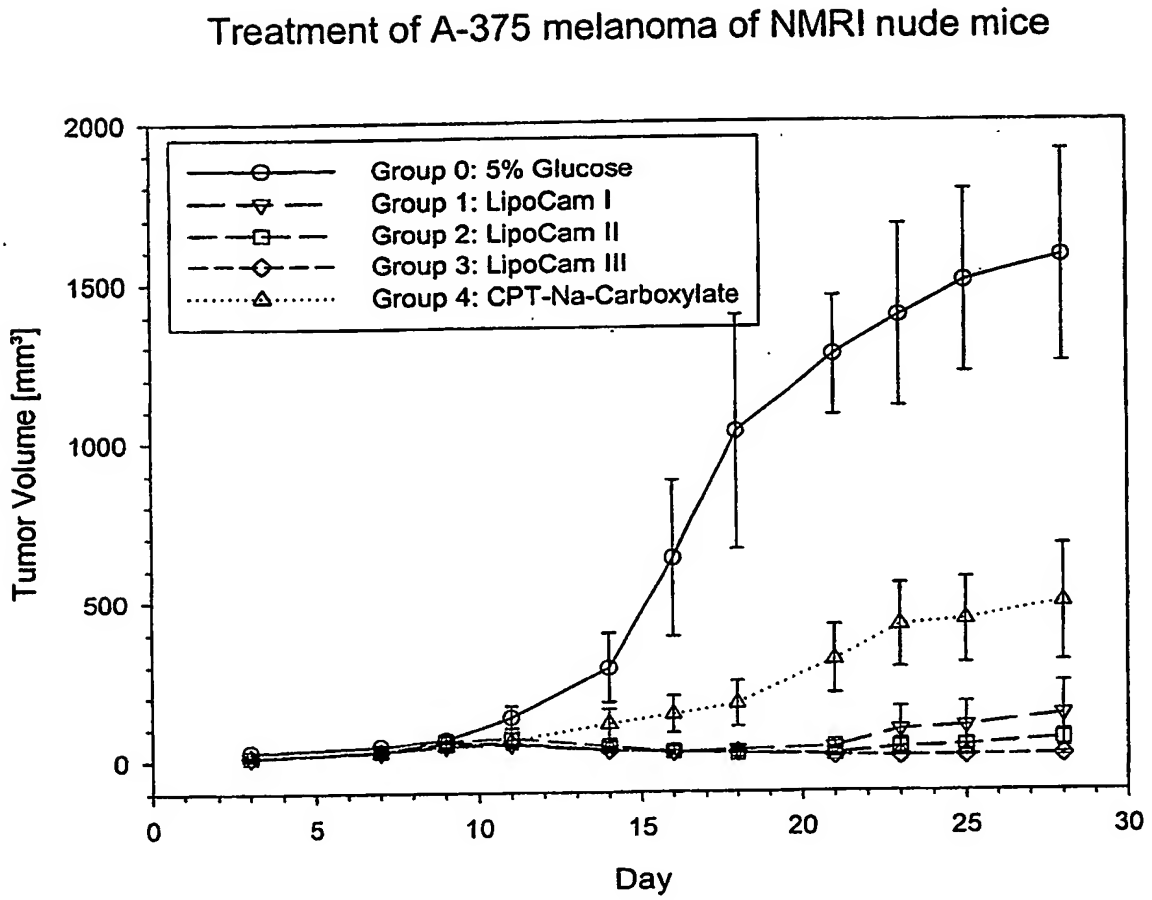
Treatment of A-375 melanoma of NMRI nude mice
Group 0: 5% Glucose
Group 1: LipoCam I
Group 2: LipoCam II
Group 3: LipoCam III
Group 4: CPT-Na-Carboxylate
Tumor Volume [mm³]
2000
1500
1000
500
0
0
5
10
15
20
25
30
Day

Fig. 12

PCT Application
EP0306760